DNA directed assembly of nanoparticle linear structure for nanophotonics

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Assemblies of metal nanospheres have shown interesting properties for nanophotonics. Here the authors describe a method to use robust DNA multicrossover molecules to organize Au nanoparticles with different sizes to form well controlled linear chain structures with desired distance below 10 nm between the particles. Au particles with only one piece of DNA attached are purified individually. Three different sizes DNA-Au conjugates then hybridize with five other DNA strands to form the stiff triple crossover (TX) motif. The linkage position of Au on the TX motif is designed so that the Au particles are organized in a well controlled linear fashion. Precise control of the distances between the Au particles can be also achieved by design of the linkage position. This arrangement of Au nanoparticle linear structures meets the requirement to construct an efficient nanolens. [DOI: 10.1116/1.3072515]

The optical properties of metallic nanoparticles and their aggregates have attracted great interest for their application to nanophotonics. Theoretical computation shows that a self-similar linear chain of several metal nanospheres with progressively decreasing sizes and separations can work as an efficient nanolens.[1]

Previously, self-assembled DNA nanostructures have been produced to generate various patterns.[2] Using DNA to organize nanoparticles was originally demonstrated by Alivisatos[3] and Mirkin.[4] Other groups used stiff DNA motifs to organize nanoparticles in well designed fashion to form one-dimensional (1D) and two-dimensional (2D) arrays,[5–7] but a linear array of nanoparticles with decreasing sizes and precise control of distance under 10 nm has not been reported. Bidault *et al.* recently reported a plasmon-based nanolens assembled on a well defined DNA template.[8] However their method still uses DNA duplex as the template, so the orientation and distance between nanoparticles is hard to control. Lithography has also been utilized to fabricate nanophotonic structures. However it is very hard to produce nanospheres by top-down methods. Here we describe a method to use a rigid DNA motif (triple crossover molecule) to organize Au nanoparticles to realize the arrangement: well controlled linear structure with desired distances. The persistence length of duplex DNA in solution is about 50 nm.[9] The DNA triple crossover molecule which contains three helical domains is much more rigid and has been used to constructed 2D and 1D arrays[10,11] over relatively large length scales. Thus we reasoned that the triple crossover should work as a rigid linear template.

Here we design a nine-turn long TX motif that consists of eight DNA strands as shown in Fig. 1. The DNA sequences were designed with the SEQUIN program[13] and listed in Table I. Three strands (labeled as 2, 5, and 7) are thiol modified at the 5 ft end to allow the linkage to Au particles. Au particles with only one piece of DNA attached are individually purified by agarose gel electrophoresis. Three DNA-Au conjugates then hybridize with five other DNA strands without Au linkage to form the TX motif. We can leave a breaking point on the DNA backbone and use thiol ended DNA at any desired position. By changing the location of thiolated strands in the TX design, we can control the position of Au attachment, and thereby control the distance between Au nanoparticles. One TX motif can template three different sizes of Au nanoparticles, for example 5, 10, and 15 nm. With properly designed sticky ends, two TX motifs can be linked together with double cohesion to assemble six nanoparticles into a symmetric chain. A computation study predicts that these six particles in a centrally symmetric chain will exhibit extremely high field enhancement.[1]

First, the formation of a hybridized DNA complex without Au particles is demonstrated by nondenaturing gel electrophoresis. DNA strands were purchased from Integrated DNA Technologies, Inc.[14] and purified by denaturing polyacrylamide gel electrophoresis (PAGE). The TX DNA motif was formed by mixing an equal quantity of each strand designed in the complex at a concentration of 1 μM, as estimated by OD260, in 1×TAE/Mg buffer (20 mM tris, *p*H 7.6, 2 mM ethylenediaminetetraacetic acid (EDTA), 12.5 mM $MgCl_2$). The DNA motif was analyzed by nondenaturing gel electrophoresis with 10%nondenaturing PAGE gel running in room temperature in 1×TAE/Mg buffer. On the gel in Fig. 2, lanes A and B are the 50 base pair (bp) ladder markers. Starting from lane 1 to 8, in each lane, we add one more DNA strand. Lane 1 is strand 1, lane 2 is the annealed mixture of strands 1 and 2, and lane 8 is the annealed mixture of all 8 strands. There is a clear single band in lane 8 and no multimerization or breakdown products which mean these eight DNA strands are hybridized to form a stable DNA complex.

Au colloids with diameters of 5, 10, and 15 nm (purchased from Ted Pella Inc.) were stabilized by incubation with bis(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (Strem Chemicals Inc.). This salt (20 mg) was added to the gold colloid solution (100 ml; ≈1 outside di-

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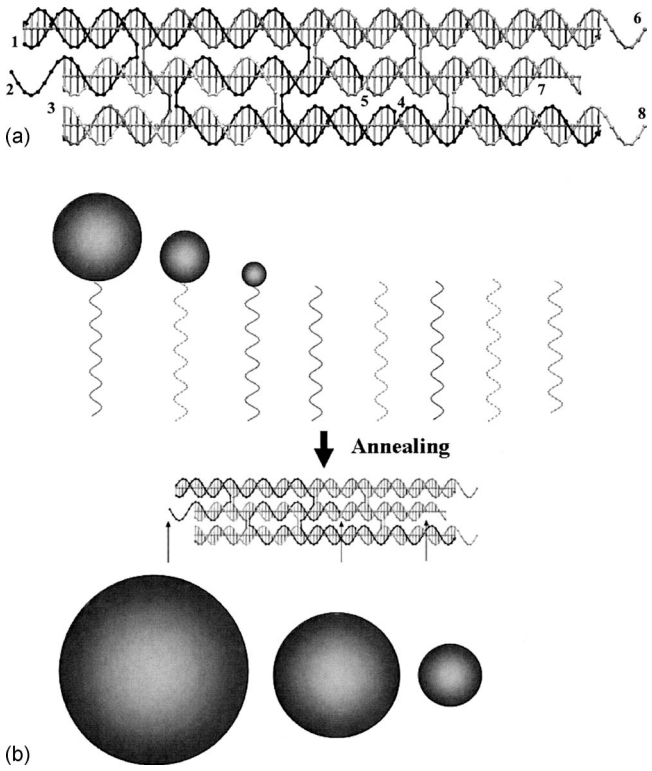

FIG. 1. (a) Structure of DNA triple crossover template. Strands are labeled at the 5′ by numbers. Strands 2, 5, and 7 have thiol group at the 5′ end. (b) Three prepurified Au-DNA conjugates will hybridize with other five DNA strands to form the DNA motif. Thin black arrows point to the Au and DNA linkage position.

TABLE I. The sequences of DNA strands used in the experiment.

Strand 1: 5′ CAGGCA TCAGA CATAC GG ACAGC CGATGGTC CGACCGCA AGCGT GGAGT GGCAT
CGT AAGTC ACATT CAA TACGG ACAAG TAACG ACACT GCTCG TTC
Strand 2: HS-5′-TTTT TTTT CCAGC AATCG CA CC GTATG TCTGA TGCCTG
Strand 3: 5′-CATCT GTTGA CC TATCC TGCTACGC CCGCTACA CCAACTGT TCACTTGG ACTCC
TGCGATTGCTGG
Strand 4: 5′-AATGT CACTT ACG ATGCC TGTAGCGG GCGTAGCA CCAAGTGA ACCAGTTGG ACTCC
TGATGTCT
Strand 5: HS-5′ TTTTTTTTACGCCAGT G GTCAT CTGGT ATCGG ACGCT TGCGGTCG GACCATCG
GCTGT GGAGT GGATA GGTCA ACAGATG
Strand 6: 5′-TTGGACG ACG GTCAA GCGAC TGCAG CATAC GCGCA CCTCA CCGTA TTG
Strand 7: HS-5′ TTTTTTTTACCA GCGTA CAGCC TCAGG ACTGGCGT AGACATCA CCGAT ACCAG
ATGACC TGCGA GTATG CTGCA TTGAC CGT
Strand 8: 5′-GTCGCAT GAA CGAGCAACGT CGTTA CTTGT GGCTG TACGC TGGT CGACAC

ameter at 520 nm), and the mixture was shaken overnight at room temperature. NaCl (solid) was then added slowly to this mixture while stirring until the color changed to light purple. The supernatant was carefully removed with a pipette after centrifugation, and the Au nanoparticles (NPs) were resuspended in 1 mM phosphine solution (5 ml). Methanol (5 ml) was then added and the mixture was centrifuged and the supernatant was removed. The Au NPs were resuspended in 2.5 mM phosphine solution (2 ml). The concentration of the Au NPs was estimated from the optical absorbance at 520 nm. Phosphine coating gives a net negative charge on the particle surface, which stabilizes the Au NPs in high concentrations of particles.

The thiolated DNA strand was purchased from IDT and purified by denaturing PAGE. The thiolated DNA strand was incubated overnight with Au NPs capped with phosphine in a ratio of 1.0:1.0 in $0.5\times$TBE containing 50 mM NaCl at room temperature. Different combinations of DNA/Au conjugate [particles bearing 1–4 single-stranded DNAs (ssDNAs)] were separated by 3% agarose gel (running buffer: $0.5\times$TBE, loading buffer: 30% glycerol, at 15 V cm^{-1}). Different bands were clearly visible from the red color of the Au NPs. The band with DNA/Au in a 1:1 ratio was electroeluted onto the glass fiber filter and a dialysis membrane (MWCO 10000) inserted in front of the band in the gel. The conjugate was recovered using a 0.45 μm centrifugal filter device. The concentration of this Au NP/DNA conjugate was estimated from the optical absorbance at 520 nm.

The Au particles conjugated with the DNA strand were further stabilized by adding thiolated T5 ssDNA oligomers to avoid further coagulation during annealing under high-electrolyte conditions. This stabilization was carried out by incubating a relatively high concentration (T5/Au=100:1) of ssDNA T5 relative to the concentration of DNA/Au conjugates in $1\times$TBE/Na+ buffer (89 mM tris, 89 mM boric acid, 2 mM EDTA, and 50 mM sodium chloride, *p*H 8.0).

To form the Au-NP and DNA complex, three Au-NP-DNA conjugates and other five DNA strands were mixed together at 80 °C in an equimolar ratio and were slowly cooled to room temperature in $1\times$TBE buffer containing

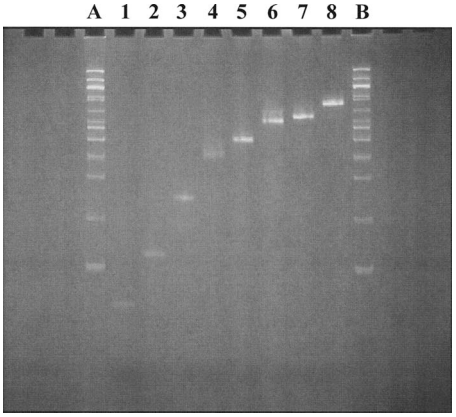

FIG. 2. Nondenaturing gel electrophoresis shows that the DNA strands hybridize to form the target DNA motif (without Au particle attached). Lanes A and B are the 100 bp ladder markers. Lanes 1–8 are annealed mixtures of DNA strands. Lane 1: strand 1. Lane 2: strands 1 and 2. Lane 3: strands 1–3. Lane 4: strands 1–4. Lane 5: strands 1–5. Lane 6: strands 1–6. Lane 7: strands 1–7. Lane 8: strands 1–8.

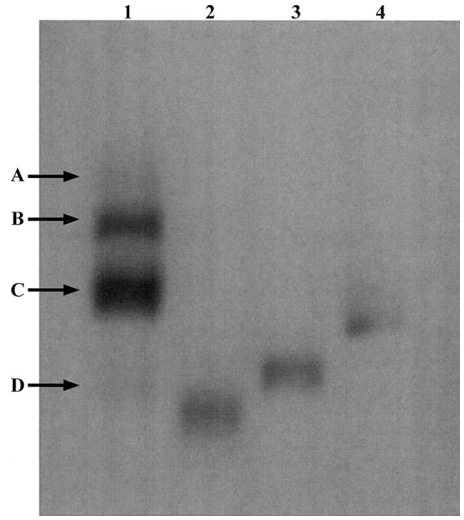

FIG. 3. Agarose gel electrophoresis of hybridization product of DNA motif with Au nanoparticles. Lane 1: Annealed mixture of three Au-DNA conjugates with other five DNA strands. Lane 2: Prepurified 5 nm Au with single DNA attached. Lane 3: Prepurified 10 nm Au with single DNA attached. Lane 4: Prepurified 15 nm Au with single DNA attached. Band *A*: target band that contains all three particles. Band *B*: partial hybridization product that contains 10 and 15 nm Au particles. Band *C*: Mixture of extra 15 nm Au particle and other small partial hybridization product. Band *D*: Extra 10 nm Au particle.

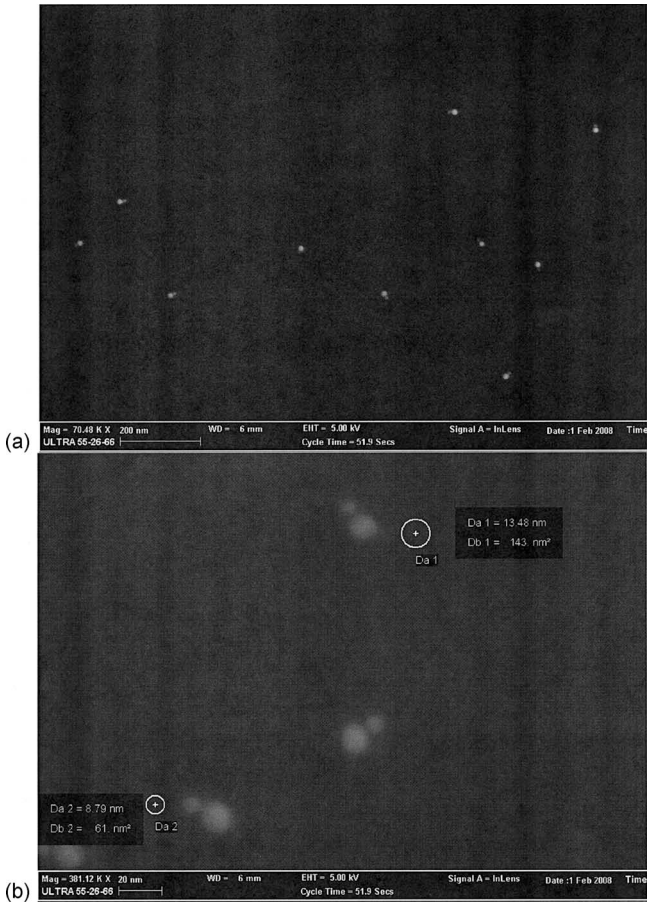

FIG. 4. (a) SEM images of band *B* sample which correspond to the partial hybridization product that contains 10 and 15 nm Au particles. (b) Zoom-in image with particle sizes labeled.

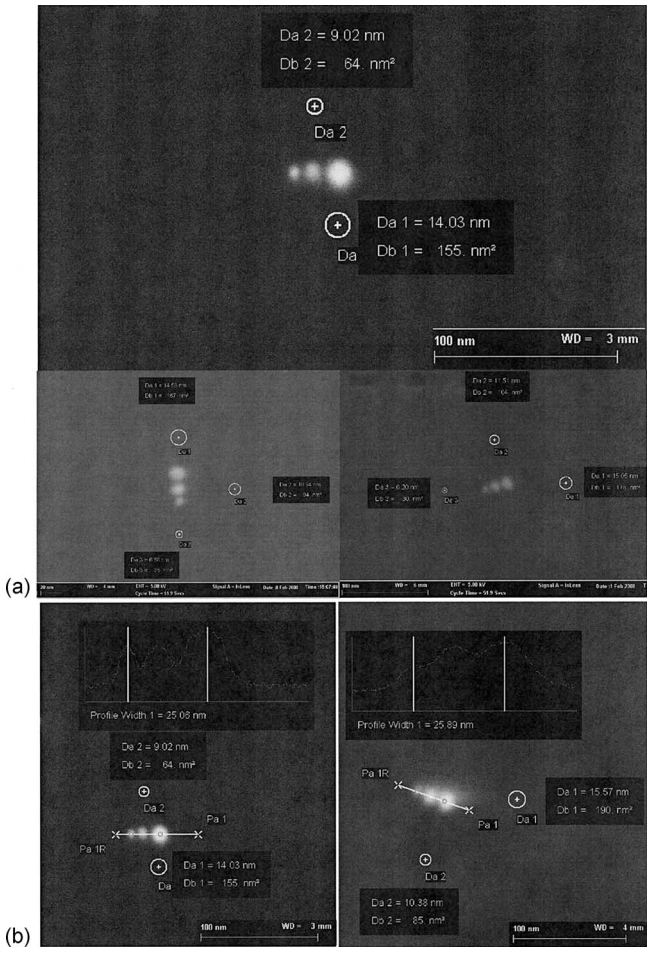

FIG. 5. (a) SEM images of band *A* sample that contains the target linear structure. 5, 10, and 15 nm Au particles form straight linear structure. The size of particles is labeled on the images. (b) Measured distances between particles are consistent with designed structure. The average center to center distance of 15 nm particle and 5 nm particles is about 25.4 nm and the gaps between particles is about 2.5 nm.

50 mM NaCl. This assembled complex is then analyzed by agarose gel electrophoresis: 3% agarose gel with running buffer 0.5×TBE (89 mM tris, 89 mM boric acid, 2 mM EDTA, *p*H 8.0), loading buffer 30%glycerol, at 15 V cm^{-1}.

Figure 3 shows the agarose gel. Lane 1 contains the annealed mixture of three Au-DNA conjugates with other five DNA strands. Lane 2 contains prepurified 5 nm Au with single DNA attached. Lane 3 contains prepurified 10 nm Au nanoparticle with a DNA attached. Lane 4 contains prepurified 15 nm Au nanoparticle with a DNA attached. Judging from the position of single Au-DNA conjugates in lanes 2–4, we expect that band *A* is the target band that contains all three particles; band *B* is the partial hybridization product that contains 10 and 15 nm Au particles; and band *C* is the mixture of extra 15 nm Au particles and other small partial hybridization products. *B* and *D* is extra 10 nm Au particles. The total yield of the target band with chain structure (band *A*) is less than 15%, but optimization of the stoichiometry among the three Au particles linked DNA and the other five DNA strands will increase the yield. Corresponding bands

are extracted from the gel. Samples of Au-NP-DNA complex were deposited on PolyLysine treated Si surface. Images were taken by Zeiss Gemini Ultra-55 analytical scanning electron microscope. The SEM images are consistent with our expectation. Figure 4(a) is the SEM image from the band *B* sample. We can see clearly that there is only a partial hybridization product that contains 10 and 15 nm Au particles. Figure 4(b) is the zoom-in image that shows the sizes of the particles. Figure 5(a) is the scanning electron microscopy (SEM) image from target band *A*. The linear structure of the three different size Au nanoparticles is clearly demonstrated. The sizes of particles are labeled on the images. The measurement of distances between the three particles is shown in Fig. 5(b). The average center to center distance of 15 nm particle and 5 nm particles is about 25.4 nm, and the gaps between particles is about 2.5 nm; which are consistent with our design.

In conclusion, linear chains of different size Au particles with controllable distance were successfully fabricated. This structure is expected to work as an efficient nanolens. The next step will be the optical characterization of these structures. The six particle centrally symmetric linear chain is predicted to show extremely high field enhancement. This linear structure can be produced by connecting two stiff TX motifs through robust double cohesion. Our method could apply to other stiff DNA template motifs to organize other sizes of nanoparticles. For example, DNA origami[12] can be use to organize more and larger different sizes particles. Here we have demonstrated that we can use biological material as the template for the nanoscale fabrication of inorganic object in a controllable fashion. The same method could be applied to the nanofabrication of other materials.

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